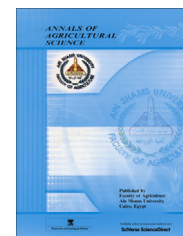




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ORIGINAL ARTICLE

Effect of citric acid, calcium lactate and low temperature prefreezing treatment on the quality of frozen strawberry



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KEYWORDS

Frozen strawberry;
Citric acid;
Calcium lactate;
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pretreatment

Abstract Texture degradation and color fading of frozen strawberries consider to be a serious problem affect their quality. Therefore, in the present study the fresh strawberry immersed in various concentrations of citric acid and calcium lactate alone or in combination for 5 min. and kept at low temperature (5 °C) for overnight before freezing or frozen directly for controlling these defects. The obtained results declared that pretreatment with citric acid dip obviously enhanced the retention of ascorbic acid and total anthocyanins content and lowered the browning index while Calcium lactate maintained the texture by reducing the drip loss and increasing the firmness after thawing. The precooling treatment did not show any enhancer affect in the quality indices of frozen berries. Compared to the other treatments, the use of 0.4% citric acid – 1% Ca lactate combination dip achieved better quality attributes, including reduction in drip loss and enhancement of firmness, retention of ascorbic acid and anthocyanin content in addition to improving in color attributes.

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Introduction

The strawberry belongs to the family Rosaceae, genus *Fragaria*, and is among the most widely consumed fruits throughout the world. Strawberries are a good source of Folate and Potassium, and a very good source of Dietary Fiber, Vitamin C, Manganese and antioxidants (Garcia et al., 1998).

The annual world production of strawberries according to FAO (2011) reached to 4,308,179 metric tonnes. Egypt occupies fourth order in the world production with quantity

reached to 240,254 tonnes produced from 5628 Hectares' (about 13,400 Feddan) represented 5.57% of the global production of strawberries and exported about 74,976 tonnes, equivalent to 58.721 million US \$ (FAO, 2011).

Strawberries are consumed mainly as fresh fruit. In addition, many other strawberry products such as juice, nectar, puree, and juice concentrate as well as jam are commercially available. Due to the highly fragile structure of strawberry fruit and their high rates of respiration, their postharvest life is relatively short (Tucker, 1993). Freezing, a simple and fast method of strawberry preservation, maintains their natural color, and nutritional value. The main components, upon which the nutritional value of foodstuff depends, are preserved best in frozen foodstuffs (Cesnauskas et al., 1998). Freezing

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strawberries is known as a good method for increasing shelf-life, but this berry undergoes quality changes throughout the whole frozen food chain. i.e. freezing, subsequent frozen storage and thawing (Kmiciek et al., 2000).

Inevitably, the freezing process and the frozen storage have negative impacts on food quality. The main physical changes that occur during frozen storage are moisture migration and ice recrystallization (Van Buggenhout et al., 2006). Both phenomena are related to the stability of products (frozen water) which affects the vegetable texture and loss of nutrients and weight if thawing drip loss takes place (Pukszta and Palich, 2007). Moreover, chemical changes in frozen vegetables are related with onset of off-odor and off-flavors (especially if vegetables were not blanched) (Nielsen et al., 2003), pigment and color degradation (Lisiewska et al., 2004) and chemical oxidation e.g. of vitamin C (Serpen et al., 2007).

It is well known that calcium plays a major role in maintaining the quality of fruit and vegetables. Increasing the calcium content in the cell wall of fruit tissue can help to delay softening and mold growth and decrease the incidence of physiological disorders (Poovaiah, 1986). Calcium dips have been employed to improve firmness and extend the postharvest shelf-life of a wide range of fruit and vegetables. With regard to strawberries, several authors have reported that CaCl_2 dips in combination with heat treatment or modified atmosphere storage and refrigeration increase calcium content and fruit firmness and delay postharvest decay (Rosen and Kader, 1989 and Garcia et al., 1996). Although CaCl_2 exerts a beneficial effect on fruit texture it has been reported to impart bitterness (Monsalve-Gonzalez et al., 1993). Organic calcium salts are an alternative calcium source and calcium lactate has been described in the literature as a firming agent for several fruit (La Cerda et al., 1999). According to Lawless et al. (2003), the bitter and salty tastes associated with calcium chloride are largely suppressed when calcium is combined with larger organic ions such as lactate, gluconate or glycerophosphate. In addition, organic acid salts of calcium such as citrate, lactate and gluconate are more bioavailable than the inorganic salts and enhance the nutritional value of foods (Labin Goldscher and Edelstein, 1996).

Besides texture and economic considerations, color is one of the most important attributes affecting consumer acceptance of food. Color stability of strawberry products, particularly after heat and light exposure, remains a challenge (Carle et al., 2001). In general, several factors are believed to affect the color and stability of strawberry anthocyanins include structure and concentration, pH, temperature, light, presence of co pigments, self association, metallic ions, enzymes, oxygen, ascorbic acid, sugar and their degradation products, proteins and sulfur dioxide (Mazza and Miniati, 1993 and Rhim, 2002). The stability of anthocyanin and phenolic content was influenced by several factors (Jiang, 2000). Among them, polyphenoloxidase plays an important role in the degradation of anthocyanin and phenolic content. Citric acid has been used extensively for the inhibitory activity on polyphenoloxidase and the anti-browning activity in minimally processed fruits and vegetables. Citric acid extracts have a double inhibitory effect by chelating copper at lower pH (Altunkaya and Gokmen, 2009). Sistrunk and Moore (1979) mentioned that bright red color of strawberry is related to acidity. High acidity and low pH stabilize strawberry color by inhibiting polyphenoloxidase during frozen storage and thawing.

Therefore, the aim of this study was to investigate the possibility of reducing the undesirable changes in color and texture of frozen strawberry by prefreezing treatments of citric acid and Ca lactate either frozen directly or after cooling for overnight at 5 °C before freezing.

Materials and methods

Materials

- Strawberries (*Fragaria ananassa*) of season (2012) used in this study were purchased from the local market in Giza governorate, Egypt.
- Chemicals used in this study were of analytical grade and purchased from El-Gomhoria Co.
- The anthocyanin standards from Carl Roth GmbH (D-76185 Karlsruhe, Germany).

Methods

Fresh strawberries were uncapped and sorted to select those full red color, being of uniform size and free of physical damage and fungal infection then washed by tap water. A mount of 1000 g berries were used for each treatment and dipped for 5 min at room temperature ~ 25 °C in various concentrations of citric acid and Calcium lactate solutions as follows:

- Citric acid solutions with concentration of 0.2%, 0.4% and 0.6%.
- Calcium lactate solutions with concentration of 0.5%, 1.0% and 1.5%.
- 0.4% citric acid solution contained either 0.5%, 1.0% or 1.5% Calcium lactate.
- Untreated sample dipped in tap water for 5 min.

Each treatment was divided into two equal portions, the first one was held overnight at 5 °C before freezing and the other portion was frozen directly. All treatments were frozen by individual quick freezing (IQF) technique in a flow freeze air blast unit at -40 °C through 20 min. Each 500 g of frozen treatments were packaged in polyethylene bags and tightly sealed. All frozen samples were stored at -18 °C for 12 month according to Egyptian Standard (ES 2368/2008). Physicochemical analysis of the frozen samples was conducted in a three replications after 24 h of thawing at 5 °C.

Analytical methods

- Total soluble solids, total titratable acidity, pH values and ascorbic acid were determined according to AOAC (2005).
- Browning index was determined according to the method of Meydev et al. (1977), as its light absorbance at 420 nm.
- Determination of Firmness (N)*: Firmness (N) measurements were performed with a universal testing machine (Comietech, Type B, Taiwan) equipped with a 2000 N load cell and operated at a crosshead speed of 20 mm/min. The force needed to compress the disk to 15 mm with a flat-plate probe (25 mm diameter) was registered. All measurements were performed at 25 °C as reported by Susana and Almeida (2008).

- (d) *Determination of total anthocyanins content*: The total anthocyanins were determined as reported by Mondello et al. (2000). Ten gm of sample was filtered through glass wool, and the pulp washed with 90 ml of a Ethanol: HCl mixture previously prepared mixing 79.7 ml of anhydrous ethyl alcohol with 20.3 ml of HCl (37%). The absorbance has been measured at 535 nm, by spectrophotometer (Jenway 6405 UV/visible, Taiwan), using 1 cm cells. The calibration was done with respect to standard curve of pelargonidin 3-glucose (Pg 3-G). The results were expressed as Pg 3-G equivalent (mg per 100 ml of sample).
- (e) *Drip loss*: Frozen samples were put over a weighted absorbent paper and let to thaw at 5 °C (Agnelli and Mascheroni, 2002). Drip loss (DL) was then evaluated by periodically weighting the absorbent paper until a constant value was reached: $DL (\%) = (W_t - W_0) / W_s \times 100$. Where W_0 is the weight of the dry absorbent paper (g), W_t the weight of the wet absorbent paper at time t (g) and W_s the weight of the frozen sample (g).
- (f) *Determination of calcium content*: This analysis was performed on frozen strawberries according to Fraeye et al. (2009). Homogenized strawberry samples were dried overnight at 105 °C. One gram of the dry sample was calcinated in a muffle furnace at 550 °C during 4 h. The residue was transferred to a 1 N HNO_3 solution and incubated at 70 °C for 1 h. The volume was adjusted to 100 mL. The Ca^{2+} concentration was measured using atomic absorption spectrophotometer (SPY9).
- (g) *Color evaluation*: For color determinations, 30 g of sample were triturated and measured with a Minolta Chroma Meter (CM – 3600d, Minolta, and Ramsey, NJ). The colorimeter was calibrated against standard white and black tiles. Measurements were performed in the CIE $L^* a^* b^*$ system, using an illuminate C. Lightness value, L^* , indicates how dark/light the sample is (varying from 0 – black to 100 – white), a^* is a measure of greenness/redness (varying from –60 to +60), and b^* is the grade of blueness/yellowness (also varying from –60 to +60). These values were used to calculate chroma: $C^* = [a^{*2} + b^{*2}]^{1/2}$, which indicates the intensity or color saturation, and hue angle, $h^*(0-360^\circ)$ obtained by $\tan^{-1} b^*/a^*$, where 0° (red – purple), 90° (yellow), 180° (bluish-green) and 270° (blue) as described by McGuire (1992). This scale is illustrated in Fig. 1 according to Coultate (2002).

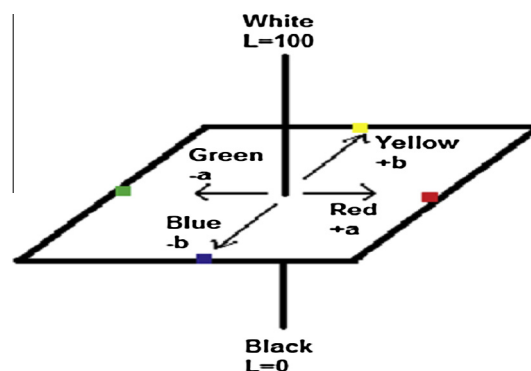


Fig. 1 Illustration of Hunter Lab scale.

is added as an acidifier in industry to most fruit and berry products and widely used in the food industries for controlling the browning. Citric acid is an anti-browning agent, which prevents polyphenoloxidase (PPO) by suppressing the food pH and binding the Cu^{2+} in an active site of PPO to form an inactive complex (Martinez and Whitaker, 1995). Since pH has a profound effect on anthocyanin stability and color expression. The control of the pH in the washing treatments has been considered an important factor (Sistrunk and Moore, 1979). Results of Table 1 showed some physico-chemical properties of frozen strawberries immersed in various aqueous citric acid solutions either alone or combined with cooling for overnight before freezing. The results declared that both citric acid treatments had a varying effect on drip loss, firmness and TSS of thawed strawberries on the other hand a slight increment in total acidity related with lowering in pH values and browning index as increasing the level of citric acid in dip solutions. The retention of both ascorbic acid and total anthocyanins was obviously increased as increased citric acid concentration while browning index was reduced due to inhibition of polyphenoloxidase as reported by Altunkaya and Gokmen (2009).

Concerning to the effect of precooling, it could be observed that the treatments of cooling – citric acid combination were lower in firmness, total acidity, ascorbic acid and anthocyanin contents compared to uncooled treatments. Furthermore, precooling treatments promoted decay in thawed berries. The pH value raised to reach 4.22 in thawed berries. Since the color and stability of anthocyanins are known to be influenced by pH. As pH increases, color fades. Higher pH will lead to the formation of the colorless carbinol pseudo-base and e subsequent chalcone molecule which have a pale yellow color (Coultate, 2002). At pH 4–6, most anthocyanins appear colorless (Mazza and Miniati, 1993). Anthocyanin content reduced by about 12.6% in cooled prefreezing berries in contrast with frozen directly. However, these results are in general accordance with that obtained by Miguel et al. (2004) who found that anthocyanin content of Assaria variety of pomegranate decrease significantly during 72 h storage at 4 °C.

Role of calcium lactate

For maintaining the texture and achieve high quality of frozen strawberry, calcium lactate was added to fresh strawberry either alone or combined with precooling. The influence of Ca lactate treatments on some properties of frozen strawberry

Results and discussion

Freezing of berries is one of the most common processes for quality preservation, because at those low temperatures deteriorative reactions are reduced to minimal rates and microorganism's growth is restricted. However, the quality of frozen berries is highly dependent on the characteristics of the raw food (Mohammad et al., 2004).

Role of citric acid

Citric acid is the most abundant organic acid in strawberry, followed by malic acid (Holcroft and Kader, 1999). Citric acids

Table 1 Effect of citric acid dip and combination with cooling on the quality indices of frozen strawberries.

Parameter	Citric acid %				Citric acid % + precooling			
	0.0	0.2	0.4	0.6	0.0	0.2	0.4	0.6
Drip loss %	36.5	35.2	32.3	33.2	38.6	37.2	35.2	35.6
Firmness (N)	13.7	12.8	13.7	11.8	12.3	10.6	11.2	10.3
TSS	5.5	5.1	5.5	5.6	5.0	4.9	5.2	5.1
Total acidity %	0.782	0.826	0.883	0.923	0.675	0.763	0.835	0.872
PH value	4.07	3.82	3.56	3.43	4.22	4.01	3.74	3.62
Browning index	0.435	0.326	0.231	0.217	0.473	0.384	0.302	0.287
Ascorbic acid (mg/100 g)	8.6	9.2	12.4	12.8	6.9	8.2	10.4	11.3
Anthocyanins (mg/100 g)	86.3	92.7	125.6	128.4	75.4	86.4	103.4	117.9

was presented in Table 2. The obtained results indicated that calcium lactate played an effective role in reducing the drip loss and improving the firmness of thawed berries. Complementing calcium treatments with precooling was more effective than other treatments. Same trends were obtained by Carle et al. (2001), they found that a combination of calcium brining and cold storage markedly improved the firmness of strawberry due to the presence of pectinmethylesterase and peroxidase activity even at low temperature and pH 4. The results declared that slight changes in both titratable acidity and pH values were found in calcium treatments either frozen directly or frozen after cooling.

Concerning to browning index, it could be observed that browning index was lowered as increasing the level of Ca lactate. Salts of calcium have been tested as anti-browning agents, Van Rensburg and Engelbrecht (1986) studied the effect of calcium salts on susceptibility to browning of avocados. Varella et al. (2007) proposed the use of minimally processed apples “Fuji” in a treatment with 1% CaCl_2 for 16 days storage at 8–10 °C.

Ca lactate pretreatments proved to be effective for increasing the retention of anthocyanin and ascorbic acid in thawed berries. The retention of ascorbic acid increased by 32.6–72.1% in Ca lactate alone treatments and by 41.1–88.4% in Ca lactate – cooling combination treatments while anthocyanin retention increased by 8.8–25.3% and 11.5–33.5% in both Ca lactate and Ca lactate – cooling combination treatments respectively such results may indicate that the firming effect is accompanied by improved water holding capacity due to a more cross linked pectin network; thus, less juice is released during thawing of frozen berries (Pukszta and Palich, 2007). Furthermore, prefreezing calcium application maintains cell turgor, membrane integrity, and tissue firmness thus extending storage life (Chaplin and Scott, 1980). These results are in line with those reported from studies carried out with strawberries

dipped in solutions of calcium salts and maintained either at 18 °C (Garcia et al., 1996) or in cold room storage (La Cerda et al., 1999).

Calcium content

Initially, Ca lactate-based prefreezing treatments clearly increased the amount of tissue bound Ca in thawed berries compared to the reference berries by three times (Fig. 1). The amount of tissue bound Ca in thawed berries that had been dipped in the Ca lactate solution and cooled increased relative to the frozen directly berries by about 15%. Treatment with 1.5% Ca lactate combined with cooling dips resulted in higher calcium contents in frozen strawberries compared to other treatments. Several authors have reported that Ca dips in combination with heat treatment or modified atmosphere storage and refrigeration increase calcium content and fruit firmness and delay postharvest decay (Rosen and Kader, 1989; Garcia et al., 1996 and Van Buggenhout et al., 2006). The employment of organic acid salts of calcium as firming agents enhances the nutritional value of foods since organic calcium salts are more bioavailable than are the inorganic salts. Organic calcium salts are already employed in therapeutic applications, such as nutraceutical dietary supplements and in the fortification of foods (Hernandez-Munoz et al., 2008).

Influence of citric acid – Ca lactate combination

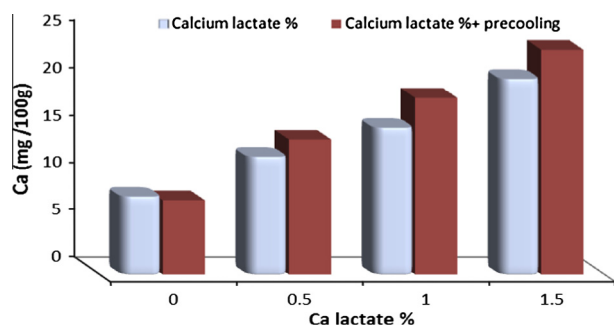
Since pH has a profound effect on anthocyanin stability and color expression and Ca plays a major role in maintaining the texture of strawberries. For achieving high quality frozen strawberries, a combination of 0.4% citric acid with different levels of Ca lactate dips was used for prefreezing treatments. The experimental results are presented in Table 3. Generally,

Table 2 Effect of calcium lactate dip and combination with cooling on the quality indices of frozen strawberries.

Parameter	Calcium lactate %				Calcium lactate % + precooling			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
Drip loss	36.5	25.4	20.5	18.7	38.6	22.8	15.8	12.4
Firmness	13.7	18.4	29.5	32.6	12.3	24.6	32.4	36.8
TSS	5.5	5.7	5.8	6.2	5.0	5.8	6.5	6.7
Total acidity %	0.782	0.793	0.822	0.834	0.675	0.793	0.825	0.847
PH value	4.07	4.12	3.87	3.80	4.22	3.93	3.74	3.65
Browning index	0.435	0.406	0.387	0.367	0.473	0.397	0.362	0.351
Ascorbic acid (mg/100 g)	8.6	11.4	13.4	14.8	6.9	12.2	15.7	16.2
Anthocyanins (mg/100 g)	86.3	89.6	104.8	108.5	75.4	96.2	112.3	119.8

Table 3 Effect of citric acid – Ca lactate combination dip and cooling on the quality indices of frozen strawberries.

Parameter	Untreated	0.4% Citric acid – Ca lactate %			0.4% Citric acid – Ca lactate % + precooling		
		0.5	1.0	1.5	0.5	1.0	1.5
Drip loss	36.5	22.6	17.2	15.7	21.5	18.4	15.4
Firmness	13.7	22.5	34.6	38.4	23.7	35.2	36.7
TSS	5.5	5.8	6.2	6.2	5.8	6.1	5.9
Total acidity %	0.782	0.815	0.885	0.892	0.822	0.854	0.873
PH value	4.07	3.82	3.65	3.58	3.76	3.65	3.54
Browning index	0.435	0.328	0.292	0.258	0.356	0.317	0.288
Ascorbic acid (mg/100 g)	8.6	15.8	18.6	19.8	13.5	16.8	16.5
Total anthocyanins (mg/100 g)	86.3	130.4	134.6	137.8	126.5	129.3	134.2

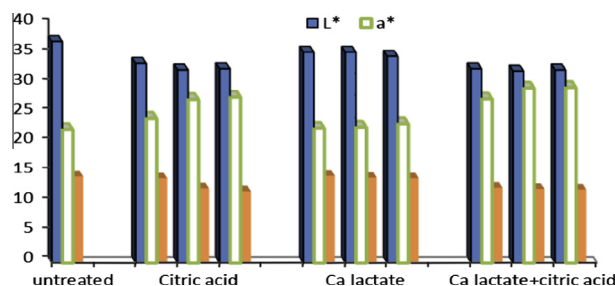
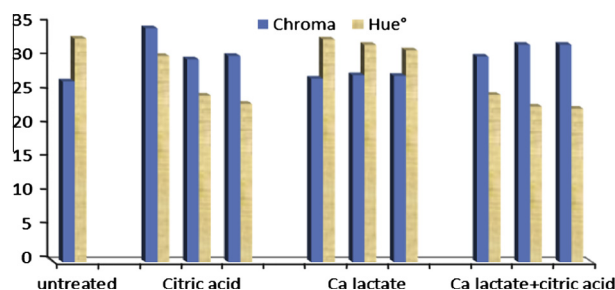
**Fig. 2** Calcium content of pretreated frozen strawberry by Ca lactate dip.

slight differences are observed between citric acid – Ca lactate combination treatments frozen directly and after cooling for overnight. Adhering 0.4% citric acid in Ca lactate dips pre-freezing treatments lead to relative improvement in quality indices, where drip loss reduced by 61–132% relative to untreated and by around 12–19% compared to Ca lactate treatments and by around 44–105% for 0.4% citric acid treatment while firmness raised by around 34–69%, 14–18% and 39–64% for untreated, Ca lactate and 0.4% citric acid treatments, respectively. A considerable reduction in browning index was also observed. The retention of both ascorbic acid and anthocyanin content was noticeably increased by 46–57% and 34–37% compared to untreated and by around 25–27% and 21–31% relative to Ca lactate treatments and by around 21–37% and 3–8% for 0.04% citric acid treatment, respectively. In a study on improving quality of home frozen strawberries, [Hudson et al. \(1975\)](#) added calcium lactate, ascorbic acid, citric acid and tartaric acid alone or in combination to a 60 (w/V) sugar syrup held in storage for up to 3 months. Citric acid gave the best color and overall rating whereas calcium lactate and ascorbic acid improved firmness, suggesting that combinations might be best (see [Fig. 2](#)).

Color analysis of thawed strawberry fruits

Color is an important factor in the perception of strawberry fruit quality. [Fig. 3](#) shows the changes in color indices of thawed strawberries as given by L^* (lightness), a^* (redness) and b^* (yellowness), while [Fig. 4](#) shows the hue angle and chroma.

The obtained results show that the untreated fruits exhibited lighter color, more yellower (highest L^* and b^* values)

**Fig. 3** L^* , a^* and b^* of pretreated frozen strawberry by citric acid and Ca lactate dip.**Fig. 4** Hue° and chroma of pretreated frozen strawberry by citric acid and Ca lactate dip.

and lower in redness (a^* value) than the pretreated frozen berries. These findings can be attributed to occurring color bleaching after storage as mentioned by [Espin et al. \(2000\)](#). As expected, the pretreatment with Ca lactate dip had a slight enhancer affect in the color indices of thawed berries. Otherwise, the incorporation of citric acid in Ca lactate dip promoted the color improvement. The pretreatment with either citric acid or citric acid – Ca lactate combination was more effective for maintaining the color of frozen berries. Accordingly, the redness (a^* value) increased by only around 4% by Ca lactate dip compared to around 24% by citric acid dip and 31% by citric acid – Ca lactate combination dip relative to untreated berries.

Generally, chroma values exhibited the same trends ([Fig. 4](#)). Since chroma reflects color brilliance or purity and is correlated with the content of anthocyanin, it will increase with pigment concentration. The pretreated frozen berries with either citric acid or citric acid – Ca lactate combination dip had higher chroma values than untreated or treated by Ca lactate alone by about 14–28%. Hue° ([Fig. 4](#)) of the untreated frozen

berries was significantly increased compared to other treatments which, in relation with a comparatively low chroma value, describe the pale- reddish color. However, these results in general accordance with that reported by Sistrunk and Moore (1979) and Altunkaya and Gokmen (2009).

Conclusion

Frozen strawberry suffers from color bleaching and texture loss. Ca lactate treatment presumably stabilizes the original structure of the strawberries and delays the changes of physico-chemical properties while citric acid inhibits the browning reaction and maintains anthocyanin content during freezing. The precooling treatment did not appear any enhancer affect in the quality indices of frozen berries. The pretreatment with combination of 0.4% citric acid – 1% Ca lactate dip achieved the highest quality attributes, including reduction in drip loss and enhancement of firmness and the retention of ascorbic acid and anthocyanin content as well as color attributes.

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